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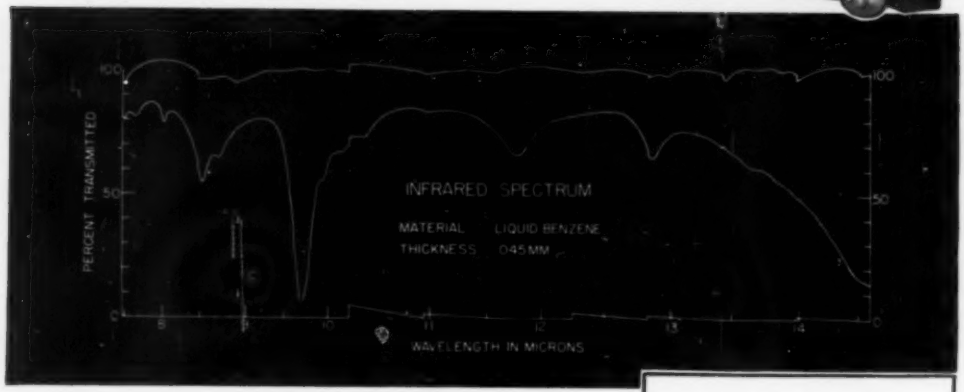

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JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

JUSTIN L. POWERS, EDITOR, WASHINGTON, D. C.

VOLUME XXXIV | *MAY, 1945* | NUMBER 5 | CONSECUTIVE No. 10

Observations on Bentonite U. S. P. and Magma Bentonite N. F.*

By Clare Olin Ewing,† Frank W. Politi,‡ and Charles H. Shackelford§

Bentonite was introduced into United States Pharmacopœia XII to permit its use as a dispersing and suspending agent in Chalk Mixture. However the use of bentonite in other drug and cosmetic products is increasing. It has, in fact, been introduced into a number of National Formulary VII formulas (1). Clays of widely different composition and properties could comply with the U. S. P. monograph and be suitable for Chalk Mixture but not for some other products in which the use of U. S. P. grade material is desirable.

The U. S. P. (2) describes bentonite as "practically colorless." Bentonite is most commonly pale buff or cream colored but some specimens have a green, gray, blue or pink cast. Practically colorless specimens are rare. All these variable shades could comply with the U. S. P. monograph, but produce finished products of widely differing appearance. We are of the opinion that U. S. P. bentonite should be pale buff or cream colored.

Bentonite is a peculiar claylike material of variable composition, first described by Knight (3, 4) in 1897–1898, although it had been known many years prior to that through its occurrence in so-called "soap holes" and had entered commerce in a small way as a detergent aid. Early reports of the Geological Survey of Canada show that it was commonly used at the Hudson Bay Company's posts for washing blankets and other woolens (5).

It was originally called "taylorite" after its discoverer in Wyoming. Shortly thereafter, this name having been pre-empted by another mineral, its name was changed to bentonite because of its first discovery in the Fort Benton formation of the Upper Cretaceous of Wyoming. These beds, while largely in Wyoming, extend also into the South Dakota Black Hills. Deposits occur also in other Rocky Mountain states and north into the Canadian Rocky Mountain provinces. Although enormous beds are reported in the Southern Appalachian states (6) they do not appear to have been exploited commercially to any great extent, possibly because they do not possess the desirable characteristics of the true Wyoming type. Bentonite deposits have been reported also from China, France and Mexico.

The consensus of expert opinion appears to be that no true Wyoming type bentonite has been discovered in this country east of the Rocky Mountains. Actually, there is considerable variation in different beds in the Benton formation and even in different

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strata in the same beds. This has resulted in wide variations in the analysis of bentonites reported in the literature by competent authorities.

This situation is only to be expected by virtue of the geologic origin of the material. It is invariably found in regions where there are extensive lava beds or volcanic ash deposits, usually accompanied by volcanic silica. The U. S. Geological Survey has defined bentonite as "a transported, stratified clay, formed by the alteration of volcanic ash, shortly after deposition" (7). Wherry (8) postulates that bentonite precursors may have formed by expulsion from volcanoes along with corrosive gases, which attacked the particle surfaces and rendered them susceptible to rapid attack when they fell in moist climates or shallow bodies of water. Part of the soluble salts resulting from the reaction were adsorbed, the rest leached out. The character of the original rock would determine the character of the resulting clay, modified at times by base replacement, since many bentonites have base exchange properties. While this appears to be the most plausible explanation of the source of most bentonites, Ross and Shannon (9) have pointed out that some deposits have been formed *in situ* by devitrification of glassy igneous ash, of lava flows, and even of hypabyssal intrusives.

A common feature therefore of bentonite deposits is the presence near-by of alkaline and alkaline earth salts, notably sodium sulfate and to a lesser extent calcium sulfate, possibly leached out of the volcanic material during the formation of the bentonite. Spence (10) states that bentonites usually contain significant amounts of soda (a common content being one to two per cent Na_2O) that cannot readily be detected and cannot be removed even by boiling with water. He concludes that the soda is adsorbed and notes that "the most highly colloidal bentonites are found on analysis, to contain the highest percentage of soda." Ladoo (11) recognizes this in his definition of bentonite: "a series of claylike materials characterized by an alkaline oxide or alkaline earth content of 5 to 10%, fine grain size, high absorptive powers and usually very strong colloidal properties." He states also that "Bentonite probably represents no mineral of fixed composition, and its physical properties vary considerably."

Davis and Vacher (12) classify bentonites into four groups: alkali bentonites, alkali sub-bentonites, alkali earth bentonites, and alkali earth sub-bentonites. The first group, into which U. S. P. bentonite falls, is described as "containing easily replaceable alkali bases and having original properties which are not permanently destroyed by the action of sulfuric acid as they can be restored by treatment with an alkali salt followed by regulated dialysis."

In general, analyses show that bentonites consist essentially of silica, alumina and water which make up roughly 90% of the material, again roughly in the order of magnitude of 60:20:10 ratios, respectively. Other common minor constituents are combined iron, magnesium, calcium, sodium, titanium, etc. Doubt exists as to the manner in which the principal constituents are combined. Probably they are chiefly a hydrous silicate of alumina, with small but variable admixtures of hydrous colloidal alumina and hydrous colloidal silica. It probably represents no mineral of fixed composition. Ross and Shannon (9) found that it is commonly composed of montmorillonite and less often of beidellite, minerals somewhat resembling micas.

Ross and Kerr (13) by X-ray analysis showed that both principal components of bentonite, montmorillonite and beidellite are of identical crystalline nature. They consider both to consist of an isomorphous series based on $Al_2O_3 \cdot 4SiO_2 \cdot H_2O \cdot x\ H_2O$ with x equal to about 4 and with H_2O replaceable with alkali or alkali earth oxides, principally Na_2O; and Al_2O_3 replaceable by Fe_2O_3, especially in the case of beidellite. Variable amounts of other minerals may be included as impurities. This no doubt accounts for the wide color variations.

Wherry (14) points out that the particles of bentonite consist of colloidal crystalline plates; that is to say, they are of less than microscopic thinness but of microscopic breadth. This would account for their extreme swelling when moistened because their micaceous texture would permit penetration of water between the infinite number of plates. The high ratio of surface to mass "gives bentonite the greatest efficiency for adsorption that Nature ever seems to have devised" (9).

Although the U. S. P. states that bentonite will absorb eight times its volume of water, our observation is that a good specimen will swell to twelve to fourteen times its volume. Spence (15) pictures a cube swollen to 13.8 times its original volume after taking up all the water it would hold without losing its original form.

Bentonites, although varying widely in composition, have many properties in common. However, because of their variable composition, these properties are themselves variable. As a result of this characteristic we have found it necessary to adjust the

amount of bentonite used in certain formulations, basing our adjustments upon an arbitrary "viscosity number."

METHOD FOR DETERMINATION OF VISCOSITY NUMBER OF BENTONITE

Our method is wholly empirical but has proved entirely satisfactory over a twenty-year period. It is based upon the dilution of a 5% aqueous suspension necessary to produce a 10-second discharge from a standard pipette (Fig. 1). The exact procedure is as follows:

Calibration of Pipette.—Calibrate a 10-cc. volumetric pipette in the following manner:

Draw up distilled water at 25° C. in the pipette to the mark *A* at base of upper pipette stem. Let it flow out of the vertical pipette for exactly seven seconds (timed with stop watch). Now mark level (*B*) of water remaining in pipette. This should be at or slightly above the juncture of the bulb with the lower stem. Repeat this process several times to ensure correct calibration of the pipette.

Determination of Viscosity Number.—Place 95 cc. of distilled water in a 250-cc. Erlenmeyer flask, and add 5.00 Gm. of bentonite in divided portions, allowing each to settle before adding the next portion, stopper and shake vigorously to break up the lumps. Let stand twenty-four hours, shaking occasionally to ensure uniform dispersion. Bring to a temperature of 25° C. and shake vigorously for one minute. Immediately draw the bentonite suspension to mark *A* and, holding the pipette vertically, allow it to discharge back into the Erlenmeyer flask, timing the outflow to mark *B* with a stop watch. If more than ten seconds are required add distilled water in increments to the bentonite suspension, shaking for a minute and repeating the test after each addition, until the time of outflow between *A* and *B* is ten seconds. When the flow is nearing ten seconds the increments should be small, such as 1 cc.

The total amount of water in the final mixture when the time of outflow is ten seconds is designated as the viscosity number. A dilution of 5 Gm. in exactly 100 cc. of distilled water discharging in precisely ten seconds (viscosity number = 100) is the base standard. This is equivalent to the viscosity of a 50% *v/v* solution of U. S. P. glycerin in distilled water at 25° C. The advantages of glycerin solutions as reference standards for viscosity have been described by Sheely (16). A good bentonite should have a viscosity number of 100 or more. Actual deliveries over the past two years have varied between viscosity numbers of 105 and 135. Figure 2 illustrates the viscosity curves of three different U. S. P. bentonites having viscosity numbers of 106, 114 and 125, respectively. The graphs show that while low concentrations differ slightly in viscosity, in the area of the concentration of Magma Bentonite, N. F., namely, 5%, the difference in viscosity is extremely marked.

EXPERIMENTAL

Certain rigid techniques must be observed in the viscosimetry of bentonite suspensions. Bentonite consists of very fine colloidal particles that are negatively charged. Consequently electrolytes affect the character of bentonite suspensions. If added to thin suspensions they tend to cause flocculation; if added to heavier suspensions they have a thickening effect. Consequently distilled water should be used in all comparative tests. If the distilled water is added to the bentonite, it tends to form a glutinous mass. Better dispersion results if the bentonite is added gradually to the distilled water with vigorous agitation.

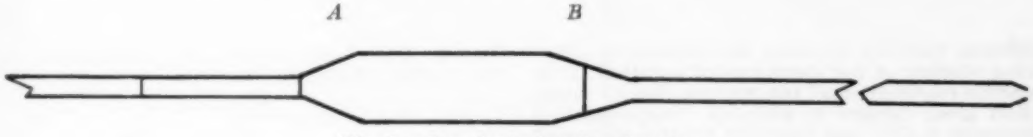

Fig. 1.—Standard viscosity pipette.

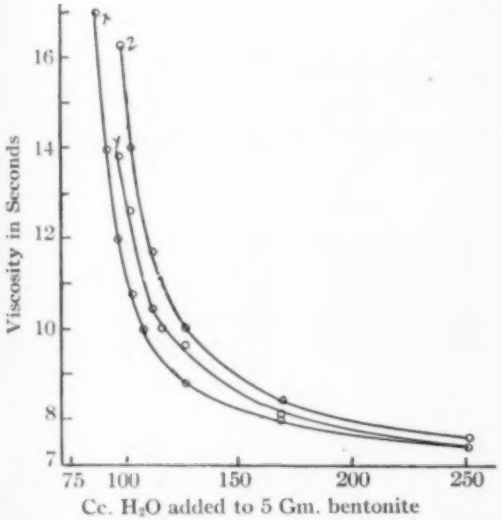

Fig. 2.—Viscosity curves of U. S. P. bentonites. Viscosity numbers: *X*, 106; *Y*, 114; *Z*, 125.

Concentrations above about 4% exhibit thixotropy, that is to say, they tend on standing to form gels that break upon agitation and reform when agitation is discontinued. In order to obtain comparable results, it is necessary to agitate the suspension vigorously until a minimum viscosity is reached. It was found that this point would always be reached in twenty-four hour suspensions by vigorously shaking about 200 cc. of the suspensions fifty times in a stoppered Erlenmeyer flask. Determinations should be made immediately and preferably should be completed within one minute's time to avoid higher readings resulting from the thixotropic setting of the gel. This is especially important in concentrations of 4% or more and when using viscosimeters of the Saybolt or MacMichael type.

The following technique was followed in making up the bentonite suspensions used in the experiments here reported: Distilled water at room temperature was placed in a Hobart Mixer of 2½-gallon capacity and the required amount of bentonite added slowly with low-speed agitation. Mixing was continued with low-speed agitation for about one-half hour until a uniform suspension was obtained. The suspensions were allowed to stand at room temperature

for twenty-four hours before testing. Two, 3, 4, 5, 5½ and 6% suspensions were made of three different U. S. P. bentonites hereafter designated as A, B and C. They had moisture contents of 6.71, 6.31 and 6.82%, respectively.

Effect of Agitation.—Suspensions of bentonite A (5%) and bentonite C (4%) at room temperature (about 25° C.) were tested with the standardized pipette (Fig. 1) after having been given a definite number of vigorous shakes. The data are recorded in Table I and illustrated in Fig. 3.

It will be noted that about 50 vigorous shakes are required to drop the viscosity to a minimum.

Effect of Temperature.—The effect of temperature on the viscosities of suspensions of bentonite of

TABLE I.—EFFECT OF AGITATION ON VISCOSITY OF BENTONITE SUSPENSIONS

Samples	Number of Shakes 0	10	25	50	75
Bentonite A, 5% suspension	23.0 sec.	20.9 sec.	20.0 sec.	19.3 sec.	19.3 sec.
Bentonite C, 4% suspension	11.6 sec.	11.2 sec.	10.7 sec.	10.5 sec.	10.5 sec.

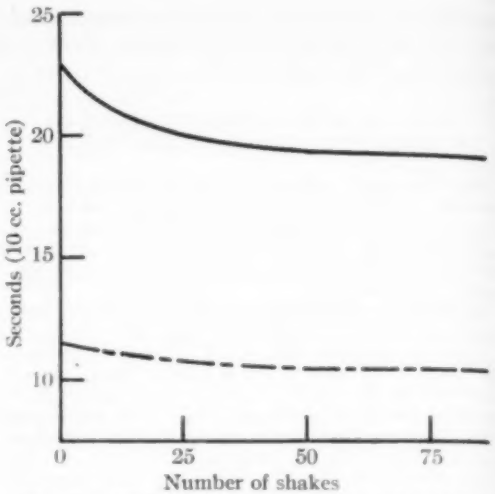

Fig. 3.—Effect of agitation on viscosity of bentonite suspensions. Sample A (5%) ———. Sample C (4%) — — — —.

TABLE II.—EFFECT OF TEMPERATURE ON VISCOSITY OF BENTONITE SUSPENSIONS

Samples	Temperatures 5° C.	10° C.	15° C.	20° C.	25° C.	30° C.	35° C.	40° C.
Bentonite A, 5% suspension	25.3 sec.	22.4 sec.	20.8 sec.	19.2 sec.	19.2 sec.	17.8 sec.	17.0 sec.	15.5 sec.
Bentonite B, 4% suspension	13.0 sec.	11.8 sec.	11.3 sec.	10.7 sec.	10.5 sec.	10.2 sec.	9.5 sec.	9.2 sec.

different viscosity numbers was studied to determine whether or not there existed a critical range wherein relatively small temperature changes might effect great changes in viscosity. Suspensions of bentonite A (5%) and bentonite C (4%) were used with the standard pipette (Fig. 1) as in the preceding

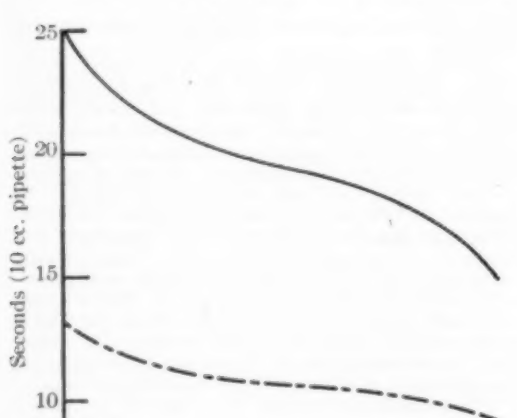

Fig. 4.—Effect of temperature on viscosity of bentonite suspensions. Sample A (5%) ———. Sample C (4%) — — — —.

TABLE III.—COMPARISON OF VISCOSITIES OF BENTONITE SUSPENSIONS WITH DIFFERENT INSTRUMENTS

Samples	Pipette (Seconds)	Consistency Cup (Seconds)	Saybolt Universal (Seconds)	MacMichael (Degrees)
Bentonite A				
2%	7.7	27.5	34.5	1.5
3%	9.0	27.7	40.3	3.0
4%	12.9	29.3	58.0	8.5
5%	19.3	32.2	105.3	24
5½%	27.1	35.0	159.7	40
6%	56.1	44.8	∝	93
Bentonite B				
2%	7.6	28.2	33.6	1.5
3%	8.5	27.9	37.3	2.0
4%	11.0	28.7	46.8	5.0
5%	14.8	29.9	66.8	12
5½%	18.9	31.7	83.4	18
6%	34.2	35.0	206.6	41
Bentonite C				
2%	7.4	27.0	33.3	1.5
3%	8.1	27.9	37.1	2.0
4%	10.5	28.1	47.6	5.0
5%	13.5	29.5	66.3	9
5½%	16.0	30.9	73.6	12
6%	26.2	34.0	140.1	29

demonstration of thixotropy. These data are recorded in Table II and illustrated in Fig. 4.

It will be noted that no critical temperature exists but that the rate of change is at a minimum in the area of the standard U. S. P. temperature of 25° C. Accordingly we chose this temperature as the standard for all instruments studied except the Saybolt Universal, for which 100° F. is the recognized standard.

recorded in Table III and illustrated by Fig. 5. The curves given by all four instruments are of the same general character and illustrate the variability in different U. S. P. bentonites. However, it will be noticed that the discharge times required by high-viscosity bentonite suspensions when using the Saybolt Universal are so high that considerable error is involved due to the thixotropic setting of the suspensions.

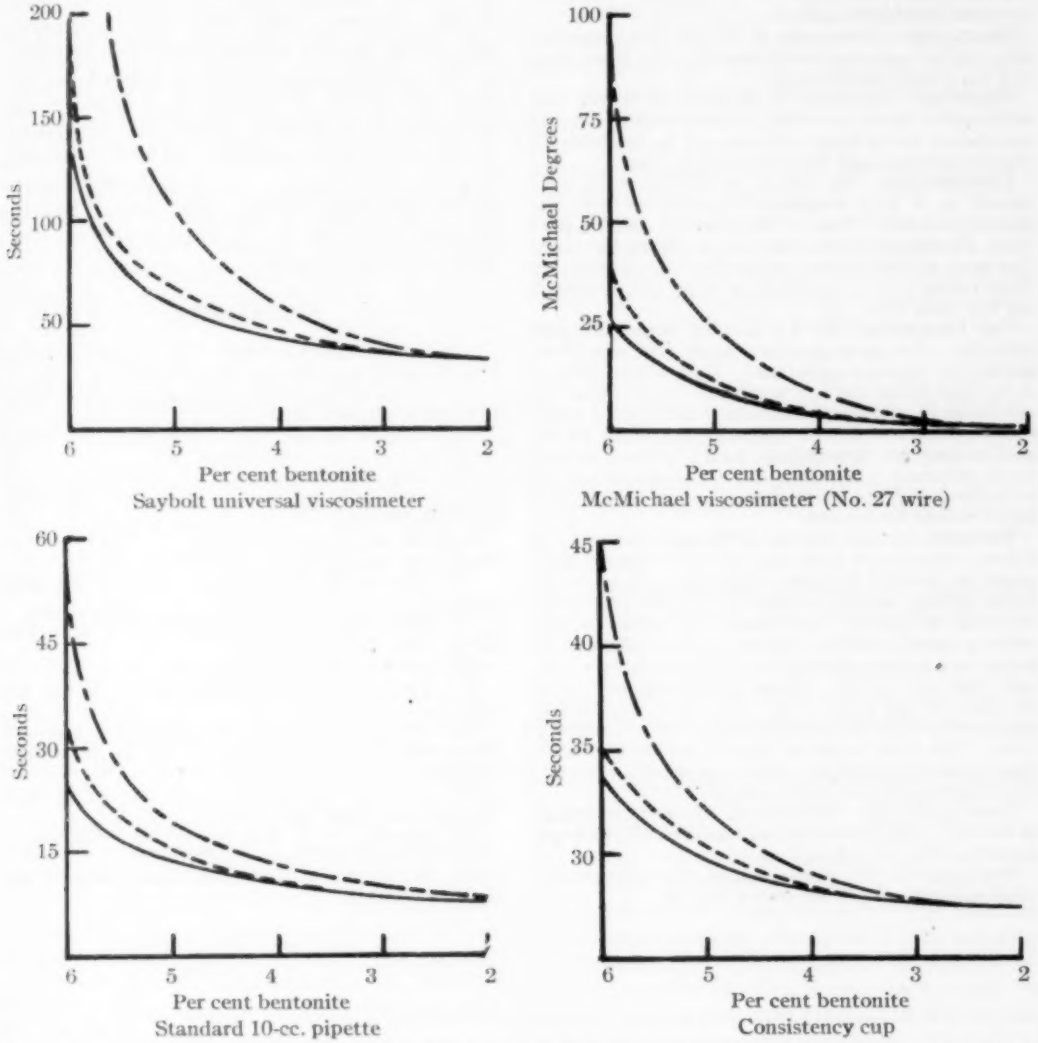

Fig. 5.—Viscosity-concentration curves showing correlation among various instruments. Sample A — — —. Sample B - - - - - - -. Sample C ———.

Adaptability of Different Viscosimeters for Bentonite Viscosimetry.—While the pipette method requires no special apparatus and is very simple, many laboratories prefer to use standard viscosimeters for much of their work. For this reason viscosity curves of suspensions of the three different bentonites were determined by use of the following instruments: Saybolt Universal Viscosimeter (seconds required for 60-cc. discharge), MacMichael Viscosimeter (degrees of torsion), Central Scientific Consistency Cup (seconds required for 100-cc. discharge) and standard 10-cc. pipette. The data are

Also, we do not consider the consistency cup sufficiently accurate for use in the viscosimetry of bentonite suspensions because of its inherent low sensitivity in the area of concentration of Magma Bentonite and the small time differentials (Fig. 5) given between bentonites of different viscosity numbers. Both MacMichael Viscosimeter and pipette readings are readily reproducible. Hence, we believe that both of the latter instruments are well suited to the standardization of U. S. P. bentonites. The pipette method, of course, has the advantage of requiring no special apparatus.

Based on these studies and on our experience with bentonite over many years, we suggest that the U. S. P. Revision Committee adopt more rigid specifications for this material. The following specifications are offered as a basis for consideration:

PROPOSED CHANGES IN THE U. S. P. MONOGRAPH ON BENTONITE

Definition.—Bentonite is a native, colloidal, hydrated aluminum silicate.

Description.—Bentonite is a very fine, odorless, pale buff or cream-colored powder, free from grit, and has a slight earthy taste.

Solubility.—Bentonite is insoluble in water, but when added to water swells to about twelve to fourteen times its original volume. It is insoluble in organic solvents and does not swell in these.

Identification.—To 100 cc. of distilled water contained in a glass-stoppered cylinder of 100 cc. capacity, add 2.0 Gm. of bentonite in divided portions, allowing each to settle before adding the next. The mass at the bottom gradually swells until after twenty-four hours it occupies an apparent volume of not less than 24 cc.

Gel Formation.—To 6.0 Gm. of bentonite, add 0.30 Gm. of light magnesium oxide and mix thoroughly by vigorous agitation. Then add to 200 cc. of distilled water and mix vigorously for one hour by mechanical agitation. Transfer 100 cc. to a stoppered cylinder of 100 cc. capacity and allow to set undisturbed for twenty-four hours. There should be no sediment. Not less than 98 cc. of gel should be formed and not more than 2 cc. of supernatant liquid should be present.

Viscosity.—Place 100 cc. of distilled water in a 250-cc. Erlenmeyer flask, and add 5.00 Gm. of bentonite in divided portions, allowing each to settle before adding the next, stopper and shake vigorously to break up lumps. Let stand twenty-four hours, shaking occasionally to ensure uniform dispersion. Bring to a temperature of 25° C. and shake vigorously for one minute. Draw the suspension up to the base of the upper stem of a 10-cc. volumetric pipette and allow to discharge to the top of the lower stem. The time required should not be less than that required to similarly discharge a 50% *v/v* solution of glycerin in distilled water at 25° C.

Loss on Drying.—When dried to constant weight at 110° C., bentonite loses not less than 5% and not more than 8% of its weight.

Reaction.—A 2% suspension of bentonite in distilled water has a *p*H between 9 and 10.

Arsenic.—Bentonite meets the requirements of the test for arsenic.

Storage.—Preserve bentonite in well-closed containers.

SUMMARY

Bentonite is a natural product that varies greatly in physical properties. The properties of the official product should be more closely defined, otherwise wide variations will result in the appearance and viscosity of the official magma and in other preparations in which bentonite is used. A description is given of an empirical method for standardizing the viscosity of bentonite suspensions that has proved useful over twenty years of commercial practice. Several changes in the U. S. P. monograph on bentonite are proposed.

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WANTED—1944 PROCEEDINGS ISSUE

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History, Literature, and Theory of Enteric Coatings*

Herman O. Thompson† *and C. O. Lee*‡

Rhazes (A.D. 850–932) deserves the title "father of pill coating" because of his use of mucilage of psyllium seed to coat offending pills and Avicenna is credited with the introduction of silver and gold pill coatings into pharmacy (1). Garot, in 1838, coated offending pills with gelatin and Warner, a Philadelphia pharmacist, was the first to manufacture pills with a sugar coating in 1866 (2).

The fact that some coatings were slower to dissolve than others was noted by Proctor (3), who stated, "The reason why pills occasionally pass through the stomach undissolved must be sought for in the state of the patient or in the composition of the pill, rather than in the nature of coating;..." In 1867 a publication on pill coatings stated that collodion protected pills from dissolving in the stomach (4). However, no effort appears to have been made to use gastric insolubility as a basis for medication until Unna, in 1884, introduced keratin-coated pills. Ceppi is credited by Yvon with the introduction of salol as a coating a few years later (5).

Enteric Coating Substances.—Since the time of Unna numerous substances and combinations of substances have been reported in the literature as being used for enteric coatings. Among the substances listed are stearic, palmitic, myristic, and lauric acids, hydrogenated castor oil, mutton tallow, carnauba wax, ceresin, paraffin, spermaceti, gelatin-formaldehyde product, keratin, gluten, casein, zein, albuminoids, decomposed scleroproteins, albumin tannate, cellulose nitrate and acetate, cellulose ethers, mastic, benzoin, tolu, sandarac, shellac, rosin, salol, tannic acid-gelatin product, cetyl alcohol, abietic acid, methyl abietate, and synthetic polymers having acid numbers and resinous properties (6).

Accessory substances reported in enteric coatings include agar, bile acids, sodium taurocholate, elm bark, alkali metal carbonates, magnesium stearate, olive oil, castor oil, lanolin, pancreatic enzymes and water-soluble agents like sodium chloride and sucrose.

Numerous combinations of these substances have also been proposed as suitable enteric coatings (6).

Patents Pertaining to Enteric Coatings.—An interesting development in the search for enteric coatings has been the synthesis of a number of polymers which have resinous properties and are insoluble in acids. Examples of such coatings are cellulose-acetate-phthalate (7), and the condensation products from compounds with reactive methylene groups and aromatic aldehydes with at least one phenolic hydroxyl (8). Further examples are the polymers resulting from vinyl butyl ether or styrene with maleic anhydride, acrylic acid with methyl acrylate, and butyl acrylate with styrene (9). Also in this classification of coatings is the resinous ester resulting from the reaction of glycerol with stearic acid and phthalic anhydride (10). An additional feature of interest in the styrene-maleic anhydride product is the inclusion of pancreatic enzymes which should assist in the breakdown of the coating in the intestines.

A very significant portion of the literature upon enteric coatings is found in the foreign and domestic patents which have been granted during the past fifteen years. No true evaluation of the subject can be obtained at the present time without a study of these patents.

An early British patent on enteric coatings was granted to Webb and Webb. It consisted of covering a capsule with a "suitable fat" and then enclosing the covered capsule in a "membranous sac" (11).

Capsules have been made enteric also by painting or coating the interior and exterior of the capsule with benzoin. Several capsules of successive sizes, if necessary, could be treated in this way and placed one within the other, the innermost capsule containing the drug (12).

A variation from an enteric coating was patented by Herrmann in which the drug was embedded in mixtures of fatty acids like stearic and palmitic acids (13).

The use of cellulose esters and ethers has drawn the attention of workers on enteric coatings for a long time. Cellulose nitrate and acetate were patented by Volwiler in 1928 (14). The use of cellulose esters or ethers in combination with organic compounds which are soluble or saponifiable in the intestines was included in an Austrian

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patent in 1931 (15). The organic compounds specified included fats, waxes, lipoids, oils, and cholic acids.

A different approach to the problem of enteric coatings was introduced by including in coating substances agents which, in the presence of moisture for a time, would so alter the structure of the coating that disintegration would take place (16, 17, 18). The method of application for this coating was an innovation. The coating materials of stearic acid, carnauba wax, petrolatum, elm bark, and agar were finely powdered and applied to tablets with an adhesive subcoating. The adhering coating material was then flamed to produce a continuous coating.

In 1931 Ellzey was granted a patent on another approach to enteric coating. The drug or medicinal agent was enclosed in a capsule and then fitted into a larger capsule which contained an innocuous alkaline material within the annular space (19).

Other coatings of interest which have also been covered by patents are ammoniacal bleached shellac granted to Wruble in 1933 (20) and a combination of abietic, oleic, and benzoic acid with methyl abietate granted to Eldred in 1937 (21).

Importance of Enteric Coatings.—The importance of enteric coatings as a form of medication was disclosed in a survey made in 1931 (23). Sixty-four professional pharmacies reported that 3.3 per cent of their prescriptions were for enteric coatings. This figure included extemporaneous and ready-made coatings. It was further disclosed in this survey that enterically coated prescriptions equaled or surpassed in frequency prescriptions for such preparations as emulsions, suppositories, hypodermic tablets, wafers, and soft elastic capsules.

The uses of enteric coatings provide a further indication of their importance. The following uses can be given for them: (*a*) to prevent gastric digestion or decomposition of the drug, for example glandular products and pancreatin; (*b*) to prevent nausea and vomiting by the drug, for example emetine, atabrine, stilbestrol, and sulfa drugs; (*c*) to prevent dilution of the drug before reaching the intestines, for example intestinal antiseptics and anthelmintics; (*d*) to prevent hindrance of gastric digestion by the drug, for example alkaline medicaments; (*e*) to give delayed action of the drug, for example sulfa drugs, aspirin, and barbiturates; (*f*) to provide a simple means of administering drugs for use in the intestines.

Fantus (22) listed six forms by which intestinal medication was possible. These were as follows: (*a*) enteric coatings, (*b*) chemical modification of solubility, (*c*) adsorption, (*d*) solution in oil, (*e*) colloid form, and (*f*) duodenal tube administration. Enteric coatings are probably more widely used than any or all of the alternatives.

A study of catalogue listings of pharmaceutical manufacturers, likewise, indicates the importance and extent of use of this type of medication. Specially developed coatings with trade names are offered for a variety of inorganic and organic chemicals in addition to glandular products and various combinations of drugs. These substances include sulfa drugs, barbituric acid derivatives, hormones, analgesics, antiseptics, and enzymes.

The renewed interest shown in enteric coatings, as evidenced by publications and patents which appeared during the years 1928 to 1940, is further indication of the importance of this class of pharmaceutical preparations.

The Theory of the Disintegration of Enteric Coatings.—A study of enteric coatings reveals that their disintegration is usually dependent upon the following factors: (*a*) the difference in *p*H between the stomach and intestines, which means that an alkaline medium is necessary. Synthetic polymers of definite acid numbers exemplify coatings of this class (7, 8, 9); (*b*) enzymatic hydrolysis in the intestines. The use of oils and fats which are saponifiable in the intestines exemplify these coatings (15, 24); (*c*) other chemical reactions such as occur between tartaric acid and sodium bicarbonate (17)—this principle has evidently not been used extensively; (*d*) time disintegration made possible by the use of hygroscopic substances which in the presence of moisture swell and cause the coating to fall apart (17); (*e*) disintegration by the use of agents like bile and cholesterol (15, 25).

Methods of Testing Enteric Coatings.—Much ingenuity has been displayed in the various means described for testing the efficiency of enteric coatings. All of these resolve themselves into *in vitro* or *in vivo* testing.

Methods *in vitro* have consisted essentially of the immersion of the coated medicaments in liquids of approximately the composition of the gastric and intestinal juices. The artificial juices suggested by Toplis (26) have been widely used by other workers (18, 24, 25). These tests are usually carried out at body temperature.

Various approaches have been made to the mechanical factors of digestion from "vigorous shaking" (28) to no agitation. Other workers have used controlled, moderate agitation such as rotating the coated

medicament in liquids contained in tubes mounted on holders at the rate of 1.5 r. p. m. (24). Other rates of agitation have been 12 r. p. m. (30) and pendulum-like oscillations at the rate of 16 per minute (25).

The role and importance of agitation have been more or less disregarded in some cases. The fact that agitation is important is attested by Schiffmann and Mirimanoff who found in tests that the presence and degree of agitation had great effect on the time of disintegration of pills and coatings (27).

A later method of testing *in vitro* consisted of warming the testing solutions to body temperature by passing them through a glass coil immersed in a bath. The solutions warmed in this manner passed over the coated pills being tested at the rate of 100 cc. per hour. Air was admitted to the chamber simultaneously with the fresh solutions to provide some agitation to the coated pills (31).

In contrast to the methods providing agitation is the method of Brenner which consists, briefly, of placing the coated medicament on a wire gauze supported on a glass vessel and immersing the whole in another glass vessel so as to cover the tested material about one centimeter with the testing solution (29). No agitation occurred other than that provided by heat and circulatory displacement.

Measures of Disintegration in Vivo.—Many studies have been made to determine the site of the disintegration of enteric coatings in the body. Ipecac in capsules or pills has been coated and the production of emesis was taken as evidence of disintegration in the stomach (26). The use of calcium sulfide which resulted in the eructation of hydrogen sulfide likewise indicated disintegration in the stomach (32). Potassium iodide, methylene blue, and sodium salicylate have been reported as useful in determining disintegration of coatings (32, 33, 34). The presence of iodides and salicylates can be determined in the urine by appropriate tests and methylene blue imparts color to the urine. Recovery of pills in the feces has been reported as the best means of determining whether disintegration has occurred (33). The use of barium sulfate pills or tablets and the determination of their course and place of disintegration by means of the x-ray and fluoroscope have been reported often (24, 34, 35). Reduced iron has also been reported as being suitable for making radiographs (29). Combinations of materials such as barium sulfate and sodium salicylate (34), calcium sulfide and methylene blue (32), and methylene blue, peppermint oil and carbonated water have been used (36). The combination with peppermint oil should be followed by periodic draughts of carbonated water which will produce the taste and odor of peppermint by eructation. A new and different method of testing enteric coatings has been described in which artificial radioactive sodium chloride was used. The position of the enterically coated radioactive sodium chloride can be located in the body by means of the Geiger counter. Moreover when the coating breaks the activated sodium chloride may be detected in the blood stream by the same means (37).

Contradictions and Lack of Standards.—Much of the contradiction found on the efficiency of various substances and coating materials can be traced to such factors as the following: (*a*) the failure to use uniform and standardized coating materials; (*b*) a lack of a standardized procedure of applying the coatings; (*c*) the failure to use a standard method of testing the coatings. Schiffmann and Mirimanoff stated that the disintegration of coated pills depends more upon the manner of coating than upon the composition of the coating (27). This serves to stress the importance of a standard method of coating.

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A Study of Enteric Coatings*

By Herman O. Thompson† and C. O. Lee‡

The physiological factors of the stomach and small intestine are of prime importance in a rational approach to a study of enteric coatings, because by definition an enteric coating must resist the action of the stomach and disintegrate in the intestines.

The normal stomach after a test meal has a *p*H of 1.67 and even when all the acid is combined with protein, the *p*H is equal to 3.0 (1). The stomach is chiefly the site of proteolytic digestion (2). Lipolytic action is confined to emulsified fats (3). Relative to the duration of gastric digestion, it is usually agreed that following an average meal the stomach empties within four or five hours (2, 3).

Best and Taylor (4), in a discussion of the relative importance of chemical and mechanical factors in digestion, point out that though the chemical aspect of digestion is now held to be the most important, the mechanical factors must not be overlooked. This view supports the fact that agitation is important in testing the disintegration rate of coatings.

Long and Fenger (5) state that the human small intestine varies from slightly alkaline to distinctly acid and that the reaction found is simply an equilibrium resulting from the acid chyme and the alkaline juices of the duodenum. Howell (1) states that from observations on dogs, with a meat and milk diet, the *p*H of the duodenum during digestion varied from 3.8 to 6.6, that the jejunum reaction was on the acid side, but the ileum did not drop below a *p*H of 7. He likewise states that fats and carbohydrates would be expected to give a greater tendency toward an acid reaction. All classes of digestive enzymes are found in the intestine (2). It is reported from observations on a patient with a fistula at the end of the small intestine, that food begins to pass into the large intestine in from two to five and a quarter hours after eating and that it requires nine or more hours, after food has entered the stomach, before the last of the meal passes the ileocecal valve. Bodansky (2) states that the small intestine, especially the lower duodenum and the jejunum, are best adapted for absorption.

The bile plays an important role in digestion by the activation of lipase, aiding the emulsification of fats, and the promotion of absorption of digested food (3). Bile renders many diverse compounds water soluble (6, 7). The conjugated bile acids retain this dissolving power even in slightly acid reaction and this extends the choleic acid principle to conditions prevailing in the upper intestine (6).

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Standards of an Efficient Enteric Coating.—From a consideration of these facts relative to the physiology of the digestive tract, it appears reasonable to set up the following standards for an efficient enteric coating: (*a*) The coating should be stable to conditions in the stomach for at least five hours; (*b*) the coating should disintegrate in the intestine, preferably, within an hour. Various workers have agreed substantially with these figures (8, 9, 10).

EXPERIMENTAL

Coating Machine.—A small coating machine was designed to coat experimental batches of from fifty to two or three hundred pills or tablets.

A reducing gear with a 48:1 ratio was mounted on a wooden platform and connected with a one-thirtieth horsepower electric motor having a speed of 1725 r. p. m. The gear and the motor had multi-drive pulleys which permitted a selection of 16 different speeds. A speed of 30 r. p. m. was imparted to the coating bowl.

The coating bowl was of glass with a capacity of approximately one liter. It had a diameter of 12.7 cm. the opening being approximately 6.25 cm. It was attached to the reducing gear by being placed on a wooden base mounted on the gear. The bowl fitted into a depression cut in the middle of the wooden base and was held in place by a collar which slipped over the bowl opening. The collar was then attached to the base by means of hooks and small springs which held the bowl securely in place.

A sprayer for applying coating solutions was made from the spraying nozzle of a small sprayer such as is commonly used for insecticides. Compressed air, dried by passing through a calcium chloride tower, was used to force the coating solutions through the nozzle. Reasonably viscous solutions, in this way, could be applied in a fine spray to the pills and tablets being coated.

The coatings applied to the pills and tablets were dried by using a jet of compressed air, which was also dried by passing through a calcium chloride tower. In the case of sugar coating an electric drier was used. Figure 1 is an illustration of the coater.

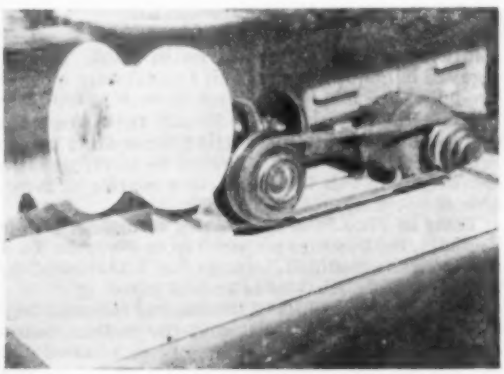

Fig. 1.—Coating machine.

Equipment for Testing in Vitro.—A water bath twenty-four inches long by eighteen inches wide by eighteen inches high with a glass front for easy viewing was maintained at body temperature, 38° C., by electric heaters and a dry cell relay.

In order to hold the samples being tested, a specially designed wheel was made having an overall diameter of twelve and one-half inches. One side of the wheel was cut out in such a manner that rubber-stoppered vials could be inserted into and held by the cut-out during the test. This wheel was made of wood and was water proofed to prevent warping by being heated in melted paraffin for several minutes and then withdrawn and allowed to dry. The wheel was set in the bath, during the test, parallel to the front glass. The vials used for testing had a capacity of about 15 cc. and could easily be seen during a test. Figure 2 is an illustration of the bath wheel.

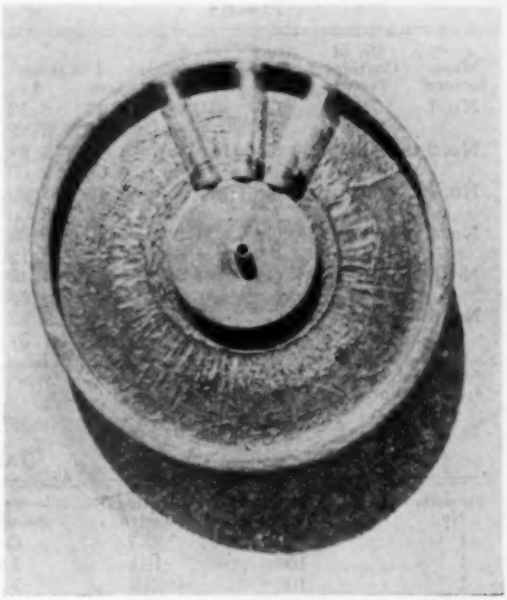

Fig. 2.—Bathwheel.

The bathwheel was rotated in the bath by means of a small stirring motor which was attached to a reducing gear. In this way a rotational speed was given to the wheel of from 1 to 2 r. p. m. This speed of rotation was calculated to produce movements approximating those of the stomach and intestines in frequency.

This equipment proved to be a convenient and satisfactory means of carrying out tests of enteric coatings *in vitro*. The wheel held eighteen or nineteen samples at one time and permitted the tests to be followed closely throughout. As the vial contents became clouded or as fresh solution was needed, the vials could easily be removed without stopping the wheel and the solutions replenished.

The testing solutions used throughout this study consisted of those described by Toplis (11). Wide use has been made of these solutions by other workers. The acid pepsin solution consisted of sodium chloride 1.4 Gm., potassium chloride 0.5 Gm., calcium chloride 0.06 Gm., hydrochloric acid (36%) 6.944 Gm., pepsin U. S. P. 3.2 Gm. and distilled water enough to make 1000.0 cc. The alkaline pancreatin solution consisted of pancreatin U. S. P. 2.8 Gm., sodium bicarbonate 15.0 Gm. and distilled water enough to make 1000.0 cc. A third pancreatin solution used in subsequent tests was ad-

justed to the desired *p*H by use of hydrochloric acid or sodium bicarbonate.

Study of Commercial Coatings in Vitro.—Six samples of enterically coated tablets, made by reputable manufacturers, were chosen for experimental observation *in vitro*. The results obtained from these tests are summarized in Table I.

Discussion.—The results obtained in Table I show that there was a considerable variation in the disintegration time of the coatings tested; however, the high figure in the case of acid pepsin solution was not a disadvantage. Assuming that the conditions of the tests *in vitro* were a reasonable approximation of conditions *in vivo*, the figures indicate that some of these coatings would not disintegrate at a desirable place in the digestive tract.

TABLE I

Manufacturer	No. of Coatings Tested	Test Solution[a]	Disintegration in Hours High	Low	Av.
No. 1	34	G	10.00	2.42	6.13
	34	I[b]	9.50	1.25	3.72
No. 2	36	G	5.00	1.17	2.57
	40	I	5.50	0.66	2.09
No. 3	32	G	17.50	3.41	8.79
	30	I	8.00	1.17	3.60
No. 4	32	G	9.50	2.50	5.58
	32	I	4.00	1.41	2.12
No. 5	28	G	9.50	1.75	4.46
	31	I	1.25	0.42	0.65
No. 6	8	G	5.50	3.00	3.92
	8	I	2.00	0.85	1.51

[a] G—Acid pepsin.
[b] I—Alkaline pancreatin.

Development of an Enteric Coating.—From a consideration of the physiological actions of the bile, it is apparent that bile possesses certain properties adaptable for use in an enteric coating. A bile preparation which suggested itself for use was iron bile salts.[1] Among its properties it is stated that the substance is acid insoluble but readily soluble in the intestine "at the optimum point for emulsification and absorption of food" (12). Sodium taurocholate (65%) was also used.

Stearic Acid U. S. P. was selected as one of the basic ingredients, because it is readily available and comparatively inexpensive. According to Hilditch (13), stearic acid was 35% absorbed by animals when administered alone.

Mutton Tallow U. S. P. has a "digestive coefficient" of 88% for human beings, which property appeared suitable for an enteric coating (13).

Balsam Tolu U. S. P. was found to increase the good storage properties of the coating and at the same time to assist in favorable behavior of the coatings.

From numerous coating experiments and tests of the coatings *in vitro*, a formula was evolved, given as Formula No. 1 in Table II, which showed good properties as an enteric coating. In order to check the necessity of certain ingredients used in this formula, a series of deletions and substitutions was made as indicated in Table II.

Technique of Coating.—Lots of two hundred pills were chosen for coating purposes. These pills possessed sugar coatings containing a red color, which assisted in establishing the disintegration time of the enteric coating by the diffusion of the red color throughout the testing solution. Warmed solutions of the coatings given in Table II were sprayed in quantities sufficient to uniformly moisten the pills, then magnesium stearate was dusted on to decrease the adhesiveness. Finally the coating was dried and molded by permitting the coating pan to rotate several minutes. Twenty to thirty applications made in this manner gave a coating of favorable appearance.

TABLE II

Ingredient	Formula Number 1	1-A	1-B	1-C	1-D	1-E
Stearic Acid U. S. P.	8.0[a]	14.0	10.0	8.0	8.0	8.0
Mutton Tallow U. S. P.	6.0	...	6.0	6.0	6.0	6.0
Balsam Tolu U. S. P.	2.0	2.0	...	2.0	2.0	2.0
Iron Bile Salts	1.0	1.0	1.0	..	..	..
Sodium Taurocholate (65%)	...	...	...	..	1.0	..
Powd. Ext. Ox Bile U. S. P.	...	...	...	..	..	1.0

[a] Quantities given in grams, solvent absolute ethanol 30 cc. and acetone 30 cc.

Tests in Vitro.—Of the coating formulas listed in Table II, the coatings prepared from Formula No. 1 and from the modified Formula No. 1-D showed the most favorable behavior in tests *in vitro*.

Several different lots of the coating formulas containing the iron bile salts and the sodium taurocholate were applied to batches of two hundred pills or modified ball-shaped tablets. Extensive tests *in vitro* were made on these coatings with the results shown in Table III.

TABLE III

Formula No.	Lots Tested	Coatings Tested	Test Solution[a]	Disintegration in Hours High	Low	Av.
1	10	235	G	14.5[b]	4.75[b]	5.96[b]
1	10	184	IA	6.0	0.25	1.55
1	10	212	I	1.75	0.08	0.53
1-D	8	136	G	7.75[b]	5.00[b]	5.45[b]
1-D	8	91	IA	7.00	0.08	1.86
1-D	8	84	I	1.25	0.08	0.48

[a] G—Acid pepsin (*p*H 1.5); IA—Acid pancreatin (*p*H 4.6 to 5.3); I—Alkaline pancreatin (*p*H 8.0).
[b] No disintegration.

Comments.—Of the two hundred and thirty-five coatings of Formula No. 1 tested in acid pepsin solution one coating disintegrated in four and a quarter hours. Of the one hundred and eighty-five coatings tested in acid pancreatin two coatings did

[1] The iron bile salts were furnished through the courtesy of Eli Lilly and Company.

not disintegrate in two hours and two in five and one-third hours.

The tests made on coatings from Formula No. 1 were plotted as shown in Fig. 3.

Storage Tests.—Several lots of coatings prepared from coating Formulas Nos. 1 and 1-D were stored in closed glass containers at room temperature for several months with no evidence of instability of the coatings. Coatings tested *in vitro* after this period of time still showed favorable behavior in the artificial digestive juices.

hours on the average, but the character of the disintegration was not as uniform as that found in alkaline pancreatin.

It has been reported that coatings of very thin character often serve as diffusible membranes through which moisture can enter or through which the contents may diffuse out (14). In the tests made on the selected commercial coatings, in a number of cases the contents were found to have diffused through the intact coating to leave nothing but an empty shell. In the coatings proposed by

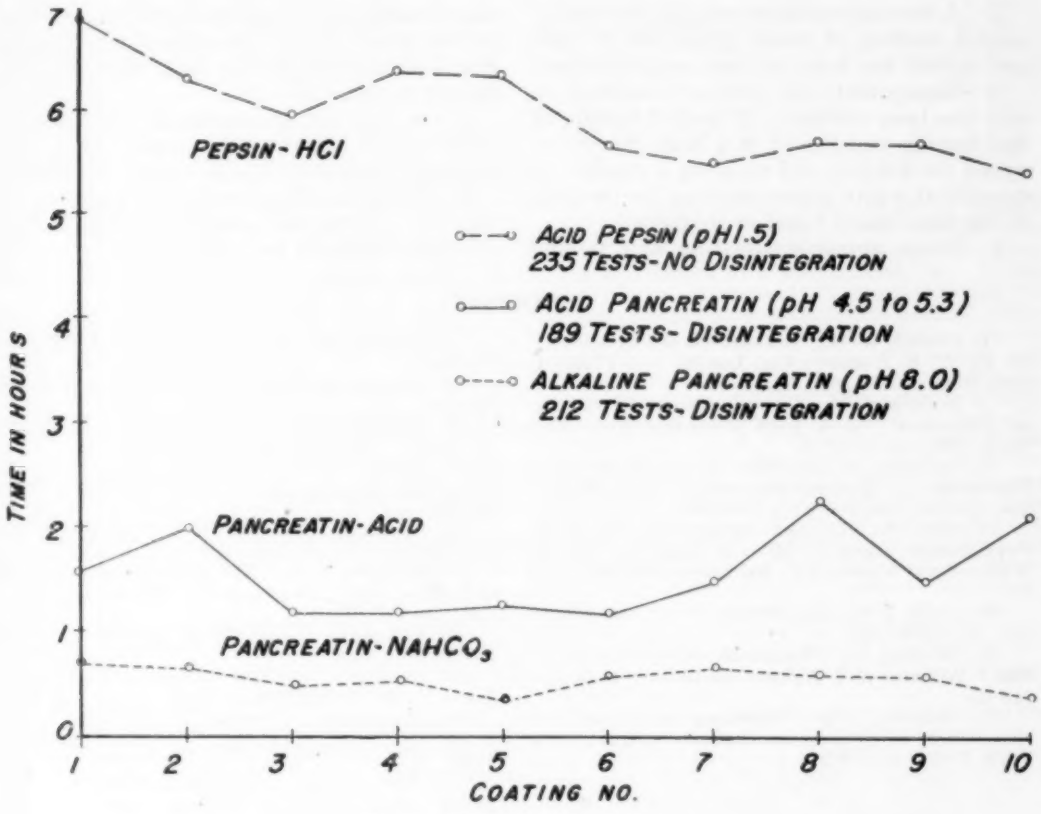

Fig. 3.—Tests made on coatings.

Thickness of the Coatings.—A number of the coatings reported in Table III were measured by means of a micrometer for thickness of the coating. They were found to vary from 0.13 mm. to 0.46 mm. Despite these wide variations the coatings gave favorable reactions when tested *in vitro*. The average thickness was approximately 0.25 mm.

Discussion.—The coatings reported in Table III showed selective behavior, qualitatively and quantitatively, in the artificial digestive juices. High resistance to acid pepsin solution was shown in all cases; however, occasionally a coating with a thin spot would show some evidence of moisture entering and softening the pill or tablet. Coatings subjected to acid pepsin solution in excess of six hours showed no visible evidence of disintegration. Coatings which were subjected to alkaline pancreatin solutions in nearly all cases showed a uniform type of disintegration with a general softening and breaking off of the coating. In the acid pancreafin solutions (*p*H 4.6 to 6.5) disintegration occurred within two

this study no evidence of diffusion was noted. The thicknesses were sufficient to adequately protect the enclosed medicament from premature release or release without actual and visible disintegration of the coating. Our results would indicate that our coatings may vary in thickness from 0.13 mm. to 0.46 mm. without having any marked effect upon their disintegration. The importance of the thickness of coatings has recently been reported to be an extremely important factor with certain combinations used as enteric coatings (9).

The appearance of the coatings was good and smooth firm coatings could be obtained by means of the coating technique which has been described.

The storage qualities of these coatings were good and showed no great change in their behavior to artificial digestive juices after as long as seven or eight months. There, likewise, were no visible changes in the appearance of the coatings after this amount of storage.

The choice of dusting powders in the preparation

of enteric coatings has been shown to be an important factor in the properties of the latter (15). Magnesium stearate was found to give a much more efficient enteric coating with mastic than did powdered talc (15). In two coatings using talc and magnesium stearate, the coating prepared with the magnesium stearate showed more favorable disintegrating properties in the pancreatin solutions. For this reason the use of magnesium stearate as dusting powder for these coatings was adopted.

Disintegration of Enteric Coatings.—The disintegration of enteric coatings has been shown, in most cases, to depend upon a single factor (16). In the development of these coatings an attempt has been made to use only those substances which aid in the disintegration of these coatings in the intestine. By the use of mutton tallow, stearic acid and bile a multiple basis of disintegration has been made. The acid value and ester content of the balsam tolu might likewise assist in the disintegration.

SUMMARY AND CONCLUSIONS

1. A coating machine suitable for experimental coating of small quantities of pills and tablets has been devised and described.

2. Equipment for testing coatings *in vitro* has been devised. A special feature of this testing equipment is a bath wheel designed for holding and rotating a number of samples at a rate approximating the motions of the alimentary canal in frequency.

3. From numerous coating and testing experiments two coatings have been devised which are of acceptable appearance, having good storage properties and favorable behavior *in vitro*.

4. A uniform and controlled method of coating was found to be important to the successful behavior of the coatings *in vitro*.

5. Disintegration has been attempted by the use of several substances which are affected chiefly in the intestines.

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WHO MAKES IT?

The National Registry of Rare Chemicals, Armour Research Foundation, 33rd, Federal and Dearborn Streets, Chicago, Ill., seeks information on sources of supply for the following chemicals:

Benzoyl choline
Butyryl choline
Nicotinuric acid
1,2-Butanediol
1-Benzyl morpholine
5-Butyl-5-isoamyl barbituric acid
Benzyl viologen
Bornyl amine
Cytosine
N,N-Dimethyl glucosamine
Fructose-6-phosphate
Galactose-6-phosphate
Hydrocupreine sulfate
Alpha (beta naphthoxy) propionic acid
Sodium alpha naphthalene sulfonchloramide
Thymine
Thitsiol
Uracil
Urushiol
Vitellin

Bacteriological and Toxicity Studies on a Wound-Healing Ointment*

By Girard W. Thomas, John C. Fardon, Stanley L. Baker, and Elton S. Cook

For the past several years our laboratories have been concerned with the investigations of natural cellular materials which influence cellular proliferation and metabolism and, in particular, with the effects of cellular injury upon the production of these materials. (For reviews see references 1 and 2.) We have also investigated the ability of certain of the respiration-promoting yeast extracts to overcome the toxic effects of phenylmercuric nitrate on the respiration of skin (3), on the growth and respiration of yeast (4) and on the growth of molds (5) and of Staphylococcus aureus (3). This work has been undertaken as an approach to Ehrlich's aim of chemotherapeutic agents with maximal desired physiological activity and minimal host toxicity. These studies have been extended to the preparation of an ointment containing a respiration-promoting aqueous-alcoholic yeast extract, prepared according to previously described methods (6), a proliferation-promoting fat-soluble fish-liver extract, and phenylmercuric nitrate. This ointment has given superior results in the healing of experimental wounds in animals (7) and human beings (8), when compared with petrolatum. A similar ointment appears to have behaved similarly to petrolatum with regard to effect on simple epithelial regeneration in a limited number of skin graft donor sites (9), but very favorable results have been reported with the ointment in the clinical treatment of burns (10, 11).

It is the purpose of the present paper to report bacteriological and toxicity studies on the ointment used in the aforementioned animal and clinical work. These experiments deal with the action of the ointment and its components on the growth of Staphylococcus aureus, Escherichia coli and Eberthella typhosa; the effect of the ointment on the growth of chick epithelium in tissue culture; and the effects of repeated topical applications of the ointment and certain of its components to animals. They were undertaken to ascertain whether, in accordance with the findings referred to in the first paragraph, the wound-healing ointment as prepared will prevent the growth of the organisms mentioned without at the same time manifesting toxicity for tissues which would tend to counteract the action of the proliferation-promoting agents.

* Received June 8, 1944, from the Research Laboratories, Institutum Divi Thomae, Cincinnati, Ohio.

EXPERIMENTAL[1]

The ointment used in the experiments, unless otherwise noted, was the same as that employed in the wound and burn work (7, 8, 10, 11), and had the following composition:

	%
Falba	2.850
Light mineral jelly	45.000
Hydrous lanolin	3.045
Amber petrolatum	45.000
Oil of thyme	0.100
Phenylmercuric nitrate (basic)	0.005
Fish-liver extract (fat-soluble)	3.000
Yeast extract (water-soluble)	1.000

The inhibitory tests were carried out by the agar platecup method (12). One-tenth milliliter of a twenty-four hour culture of the organism in nutrient broth was placed in a sterile Petri dish. Sterile nutrient agar, cooled to 42° C., was poured over the culture, swirled and allowed to harden. After solidification, a hole was bored in the agar by means of a sterile cork borer and the melted ointment was poured in. The plates were incubated for forty-eight hours at 37° C. The organisms used were Staphylococcus aureus (A. T. C. C. No. 152), Escherichia coli (A. T. C. C. No. 130) and Eberthella typhosa (A. T. C. C. No. 7251). The results of the experiments on the ointment and its components are given in Table I and, in part, in Fig. 1. Lack of space prevents reproduction of photographs of all plates. In addition, experiments were carried out using the complete ointment, but varying the concentration of phenylmercuric nitrate. Similar experiments were performed with the substitution of phenylmercuric benzoate for the nitrate. These data also appear in Table I. All experiments were conducted in replicate by two bacteriologists, working independently.

Tissue culture experiments were carried out by the hanging drop method, using implants of eight day-old chick embryo skin in a medium of beef plasma and calcium-Ringer's solution. In each experiment the skin fragment was placed adjacent to a patch of ointment within the plasma clot.

Topical toxicity experiments were conducted by applying to separate groups of rats the ointment, the ointment base, the yeast extract and, as a control, Ringer-phosphate-glucose solution. Twenty-five male rats weighing between 40 and 50 Gm. and eighteen to twenty-three days old were used for each group. A region $1^1/_2$ in. square on the abdomen was shaved and rubbed with the material being tested. The material was applied four times weekly for twenty-six weeks (total of 104 applications) and the animals were shaved from time to time as required. The weights of the animals were recorded weekly and the animals were observed with respect to local irritation, change in skin and hair texture, macropathology and general health. At the termination of the experiment, at least three from each group of animals were sacrificed, sections were made of the skin through the tested area, and the organs were examined for pathological indications.

[1] Acknowledgment is due to Dr. J. F. Kowalewski for preparing the ointment, to Dr. L. G. Nutini for the autopsies and to Mary Jane Heimbrock for technical assistance.

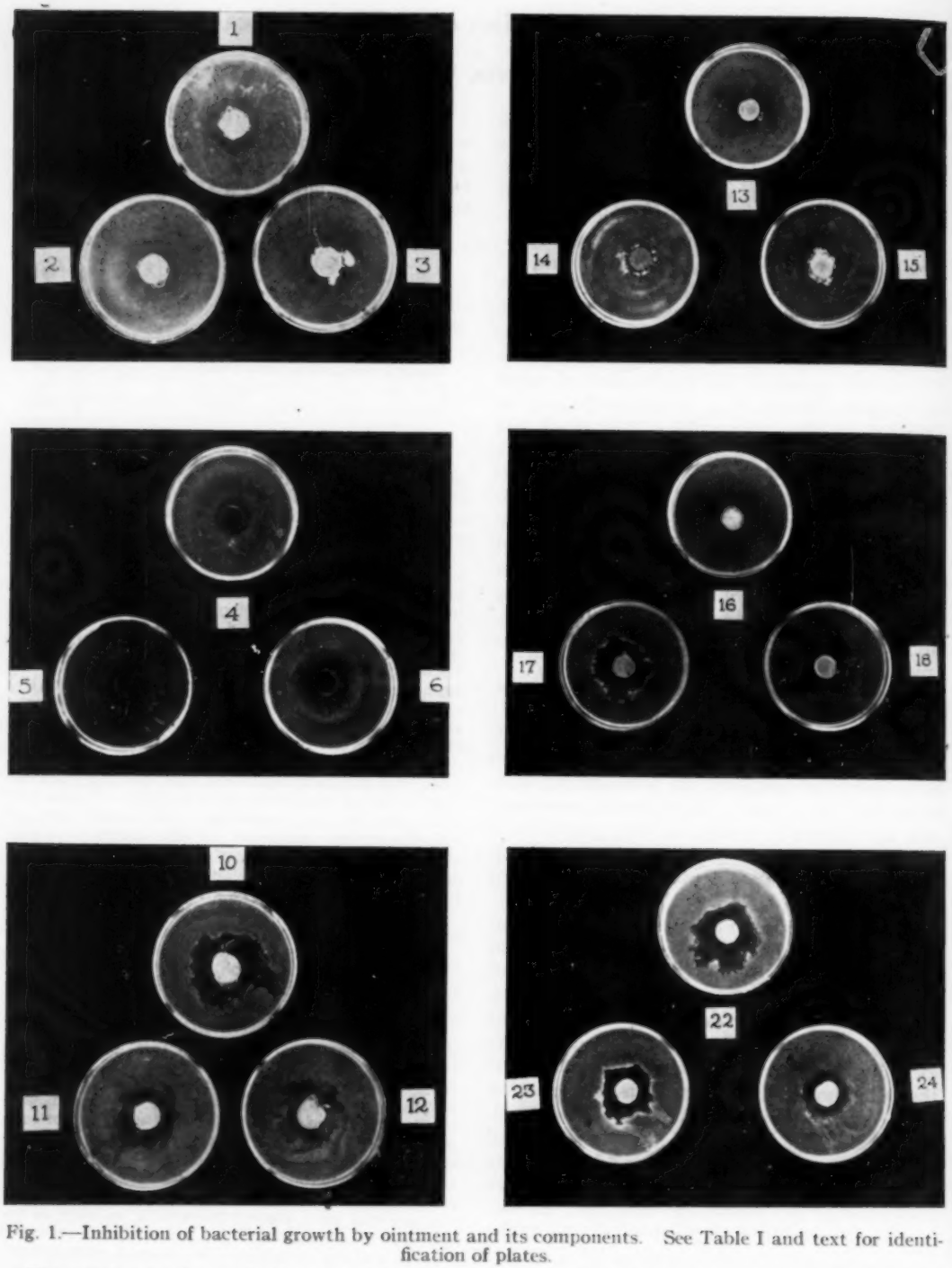

Fig. 1.—Inhibition of bacterial growth by ointment and its components. See Table I and text for identification of plates.

The yeast extract was further tested by daily subcutaneous injection for five weeks at a level of 1 Gm. per Kg. into 12 rats and by daily intravenous injection for four weeks at a level of 100 mg. per Kg. into 3 rabbits.

Results and Discussion

1. Bacteriological.—Figure 1, Plates 1–3, shows that the ointment base produced no inhibition of the growth of the organisms. Therefore the zone produced in the other experiments was due to the additional ingredients incorporated in the base. Phenylmercuric nitrate 1:20,000 (Fig. 1, Plates 4–6) shows good action against Staph. aureus and E. typhosa and a lesser action against E. coli. The fish-liver extract, 3% in the ointment base, inhibited the growth of all three organisms (Fig. 1, Plates 22–24) which is in agreement with the reports of germicidal activity of cod-liver oil. (For references see Holmes (13) and Hardin (14).) However,

in our experience, certain commercially available fish-liver oils used as concentrated sources of vitamins A and D have not always exhibited inhibitory properties under these test conditions.

Incorporation of the yeast extract into the base containing the fish-liver extract (Fig. 1, Plates 13–15) or containing phenylmercuric nitrate 1:20,000 overcame, in part or completely, the toxicity of both substances for the organisms. This is in accord with the earlier observations on yeast extract–phenylmercuric nitrate antagonism (4, 5, 6). However, the complete ointment (Fig. 1, Plates 10–12) containing both phenylmercuric nitrate and the liver extract, plus the same quantity of yeast extract which was inhibitory to either germicidal material singly, possesses as good inhibitory properties as the similar ointment without the yeast extract (Fig. 1, Plates 16–18). It might be expected that the yeast extract in the concentration employed would be unable fully to overcome the inhibitory powers of the combination of phenylmercuric nitrate and liver extract, but it is somewhat surprising to find the inhibitory powers of the complete ointment as marked as they are. This has been demonstrated repeatedly with different batches of ointment prepared from different samples of yeast extract and is dependent upon careful preparation and adjustment of the quantities of the ingredients, for improperly prepared ointments have sometimes been found to be deficient in ability to inhibit E. coli and E. typhosa.

Table I shows, in addition to the above results, that the components of the ointment base have no inhibitory activity, and addition of the yeast extract to the base, as would be expected, does not alter the picture. Ointments prepared with concentrations of phenylmercuric nitrate and phenylmercuric benzoate ranging from 2:2500 to 1:20,000 were all inhibitory, as is evident from Table I. These results are in accord with the work of Weed and Ecker (15) and Baron and DeKay (16) on the activity of phenylmercuric nitrate and benzoate, respectively, in ointment bases and indicate the compounds to be active in greater dilution than used by these authors, although the modifying effects of the other components of our ointment must be borne in mind.

2. Tissue Culture Experiments.—Forty-eight cultures were prepared and all exhibited very good growth after forty-two hours' incubation at 37.5° C., with an extensive zone of new cells invading and spreading over the localized area of the ointment, as shown in Fig. 2. The ointment manifests no toxicity for the epithelium, there being as good migration and growth into the ointment area as into the control area.

3. Topical Toxicity Experiments.—No evidence of irritation, desquamation of epithelium or change of skin texture was seen in any of the animals tested with the complete ointment, the ointment base or the yeast extract. Slight initial desquamation, which disappeared in three to five days, appeared in the animals rubbed with Ringer solution. The

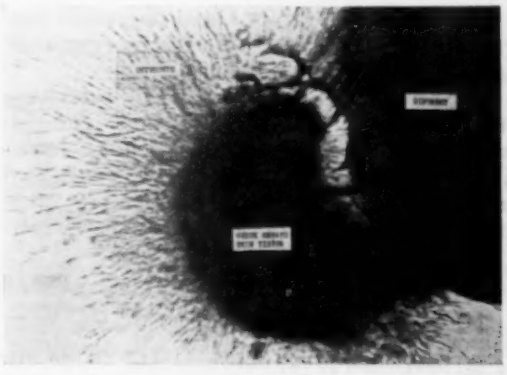

Fig. 2.—Effect of ointment on growth of chick epithelium in tissue culture.

TABLE I.—INHIBITION OF BACTERIAL GROWTH BY OINTMENT AND ITS COMPONENTS

Material	Inhibition[a] E. coli	E. typhosa	Staph. aureus
Yellow petrolatum	0 (1)[b]	0 (2)	0 (3)
Lanolin (hydrous)	0	0	0
Falba	0	0	0
Petrolatum plus falba	0	0	0
Petrolatum plus lanolin	0	0	0
Ointment base	0	0	0
Aqueous PMN[c] 1:20,000	+++ (4)	+++ (5)	+++ (6)
Ointment base plus 3% liver extract	++++ (22)	++++ (23)	+++ (24)
Ointment base plus 1% yeast extract	0	0	0
Ointment base plus PMN 1:20,000 and 3% liver extract	++++ (16)	++++ (17)	++++ (18)
Ointment base plus 1% Yeast Extract and PMN 1:20,000	0	0	0
Ointment base plus 1% yeast extract and 3% liver extract	++ (13)	++ (14)	0 (15)
Complete ointment (see text for formula)	++++ (10)	++++ (11)	++++ (12)
CO[d] with PMN 1:2500	++++	++++	++++
CO[d] with PMN 1:5000	++++	++++	++++
CO[d] with PMN 1:10,000	++++	++++	++++
CO[d] with PMN 1:20,000	++++	++++	++++
CO with PMB[e] 1:2500	++	++	++++
CO with PMB[e] 1:5000	++	++	++++
CO with PMB[e] 1:10,000	++	++	+++
CO with PMB[e] 1:20,000	++	++	+++

[a] In Table, 0 = none, + = zone of 1 mm. or less, ++ = zone of approximately 1–3 mm., +++ = zone of 3–8 mm., and ++++ = zone of 8 mm. or more. These figures are averages since uneven diffusion often leads to nonuniform zones.
[b] Figures in parentheses indicate plate numbers in Fig. 1.
[c] Phenylmercuric nitrate (basic).
[d] Complete ointment with specified germicide and concentration.
[e] Phenylmercuric benzoate.

growth response of all the animals was normal, the growth curves for the groups being superimposable within experimental error. None of the animals from any of the groups showed gross internal pathology upon autopsy, nor was any evidence of abnormality or pathology discovered upon examination of the heart, spleen, lungs, liver, gastro intestinal tract, kidneys, adrenals and reproductive glands. Sections of the skin, through the treated area, showed no macropathology. The animals receiving the subcutaneous and intravenous injections of the yeast extract likewise showed no evidence of toxicity.

The lack of toxicity of the ointment, as shown by the tissue culture and topical application methods, is confirmed by preliminary experiments (performed with the collaboration of C. W. Kreke) which indicate that rat skin treated with the complete ointment does not show a depressed respiration when measured in Warburg respirometers by the previously described method (3, 6, 17), whereas the ointment from which the yeast extract is omitted may cause a slightly lowered respiration.

SUMMARY

1. The ability, previously established, of a yeast extract to antagonize the germicidal activity of phenylmercuric nitrate has been confirmed for the germicide incorporated in an ointment base.

2. The yeast extract also antagonizes the growth-inhibitory action for microorganisms of a fat-soluble liver extract.

3. An ointment used successfully for the treatment of experimental and clinical wounds and burns, containing a fat-soluble fish-liver extract, a water-soluble yeast extract and phenylmercuric nitrate: (*a*) Inhibits the growth of Staph. aureus, E. coli and E. typhosa; (*b*) reveals no accute or chronic toxic manifestations when tested by tissue culture and topical application methods; and (*c*) supports the growth of epithelium in tissue culture.

4. The above findings, together with the earlier work, show that, although a yeast extract will antagonize the action of phenylmercuric nitrate for microorganisms and tissues, proper adjustment of concentrations and ingredients allows the preparation of an ointment which retains action against microorganisms with minimal toxicity for tissues.

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WHO MAKES IT?

The National Registry of Rare Chemicals, Armour Research Foundation, 33rd, Federal and Dearborn Streets, Chicago, Ill., seeks information on sources of supply for the following chemicals:

Aceto pyruvic acid
o-Aminobenzyl-4-methyl thiazolium chloride
Butanone-2-ol-4 and its acetate
Coenzyme II
Cytochrome C
Cascara emodin
Chrysophanic acid
Carbonic anhydrase
4- or 5-Chloro nicotinic acid
Dimethyl diacridylium nitrate
Ergothioneine
Erythrose
Estragole
Ethyl antimonyl tartrate
Ethyl β-4-morpholylethyl ether
4-Fluoro coumarin
Flavone or flavanone
Guvacine or guvacoline
Ginnol
2-Hydroxy thiolpropionic acid
Myricin
Octamethylene glycol
Psyllastearyl alcohol
p-Sulfonamidobenzamidoxime hydrochloride
Threose
Thioxane

The Synthesis of Derivatives of 5-Aminoquinoline*

By John E. Christian† and Glenn L. Jenkins‡

The successful use of quinoline derivatives in many important therapeutic applications, and the successful use of sulfanilamide and its derivatives in chemotherapy make the syntheses of quinoline analogues and derivatives of sulfanilamide of considerable interest. It is believed that compounds of this type may be of some value as active chemotherapeutic agents. In order that quinoline analogues and derivatives of sulfanilamide may be tested for such properties, compounds containing both the quinoline and sulfanilamide nuclei have been prepared.

By the following reactions, 5-acetamidoquinoline-8-sulfonic acid was successfully synthesized. The Skraup reaction on *o*-chloroaniline produced 8-chloroquinoline, which was nitrated to yield 5-nitro-8-chloroquinoline. The latter compound was treated with sodium disulfide to produce 5,5′-dinitro-8,8′-diquinolyl disulfide. This compound was oxidized with nitric acid to 5-nitroquinoline-8-sulfonic acid, the sodium salt of which was reduced with iron and acetic acid to sodium 5-aminoquinoline-8-sulfonate, and then was converted to 5-aminoquinoline-8-sulfonic acid which was acetylated with acetic anhydride and sodium acetate.

An attempt to synthesize 5-acetamidoquinoline-8-sulfonamide, a quinoline analogue of *p*-acetamidobenzenesulfonamide, by the amidation of 5-acetamidoquinoline-8-sulfonic acid, first by treatment with chlorosulfonic acid and then with ammonium hydroxide, resulted in the formation of 5 - acetamido - 6 - sulfamylquinoline - 8 - sulfonic acid.

The synthesis of 5-(*p*-acetamidobenzenesulfonamido)-8-chloroquinoline was accomplished as follows: Using iron and acetic acid, 5-nitro-8-chloroquinoline was reduced; the reduced product was condensed with *p*-acetamidobenzenesulfonyl chloride to yield the desired product.

From 5 - (*p* - acetamidobenzenesulfonamido)-8-chloroquinoline, by acid hydrolysis of the acetyl group, 5-(*p*-aminobenzenesulfonamido)-8-chloroquinoline was prepared.

By the following reactions, 5,5′-bis-(*p*-acetamidobenzenesulfonamido) - 8,8′ - diquinolyl disulfide was successfully synthesized. Using stannous chloride and hydrochloric acid, 5,5′-dinitro-8,8′-diquinolyl disulfide was reduced to 5,5′-diamino-8,8′-diquinolyl disulfide, which was condensed with two molecules of *p*-acetamidobenzenesulfonyl chloride resulting in the desired product.

In order to characterize some of these compounds, derivatives were prepared.

EXPERIMENTAL

1. 8-Chloroquinoline and 5-Nitro-8-chloroquinoline.—The procedures used were essentially those of Fourneau, *et al.* (1), and Urist and Jenkins (2).

2. 5,5′-Dinitro-8,8′-diquinolyl Disulfide.—The procedure used was that described by Bogert and Stull (3) and by Urist (4). A 93% yield was obtained. A small amount recrystallized from toluene melted with decomposition at 249–250° C. (corrected). Winter and Reinhart (5) reported a melting point of 245° C. (uncorrected) and Urist and Jenkins (2) reported 250–252° C. (corrected).

3. 5-Nitroquinoline-8-sulfonic acid and Sodium 5-Nitroquinoline-8-sulfonate.—The procedures used were essentially those described by Urist and Jenkins (2). The sulfonic acid melted above 210° C. (with decomposition). Urist and Jenkins (2) reported a melting point above 211° C. (with decomposition). A 72% yield resulted; a 90% yield of the sodium salt was obtained.

4. Sodium 5-Aminoquinoline-8-sulfonate.—A modification of the method proposed by Wertheim (6) was used. To 60 Gm. of crude sodium 5-nitroquinoline-8-sulfonate dissolved in 1200 cc. of 50% acetic acid was added 100 Gm. of iron filings (20 mesh) over a period of four hours during which time the suspension was kept at 90° C. and the stirring was continuous. The stirring and heating were continued for one hour longer, after which the mixture was cooled, filtered, and the residue suspended in water, made alkaline with sodium carbonate, heated to boiling, and filtered. From the cooled filtrate the sodium salt was collected and recrystallized from 70% alcohol; yellow needles; yield 45 Gm. (85%).

Anal.—Calcd. for $C_9H_7N_2O_3SNa$: Na, 9.30%. Found: Na, 9.26%.

5. 5-Aminoquinoline-8-sulfonic Acid.—Sodium 5-aminoquinoline-8-sulfonate (45 Gm.) was dissolved in the least amount of hot water necessary and the solution made slightly acid with dilute hydrochloric acid. On cooling, 33 Gm. (81%) of orange-red crystals separated and were recrystallized from water with the aid of decolorizing charcoal. The crystals melted at 300–310° C. (with decomposition). Cybulski (7) reported a melting point of 290–310° C. (with decomposition).

6. Benzylisothiourea Salt of 5-Aminoquinoline-8-sulfonic Acid.—This derivative was prepared analogous to a method described by Veibel and Lillelund (8) and Urist (4). Orange-yellow needles were obtained; m. p. 225–227° C. with decomposition (corrected).

Anal.—Calcd. for $C_{17}H_{18}N_4O_3S_2$: N, 14.35%. Found: N, 14.50%.

* Received July 25, 1944, from the College of Pharmacy, Purdue University, Lafayette, Ind.

† Eli Lilly and Company Research Fellow, 1940-1943.

‡ Dean, College of Pharmacy, Purdue University.

7. 5-Acetamidoquinoline-8-sulfonic Acid.—Dry 5-aminoquinoline-8-sulfonic acid (33 Gm.) was refluxed with 550 cc. of acetic anhydride for fifteen minutes; 27.5 Gm. of anhydrous sodium acetate was added and the refluxing continued for one hour. The precipitate which formed on cooling was recrystallized from water using decolorizing charcoal; orange-red platelets; yield 30 Gm. (77%); m. p. 353–360° C. with decomposition (corrected).

Anal.—Calcd. for $C_{11}H_{10}N_2O_4S$: N, 10.53%. Found: N, 10.62%.

8. Benzylisothiourea salt of 5-Acetamidoquinoline-8-sulfonic Acid.—This derivative was prepared analogous to a method described by Veibel and Lillelund (8) and Urist (4). White needles were obtained; m. p. 250–253° C. with decomposition (corrected).

Anal.—Calcd. for $C_{19}H_{20}N_4O_4S_2$: N, 12.97%. Found: N, 13.35%.

9. Sodium 5-Acetamidoquinoline-8-sulfonate.—To a hot water suspension of 30 Gm. of crude 5-acetamidoquinoline-8-sulfonic acid, sufficient sodium carbonate was added to make the solution alkaline and the mixture thus obtained was placed in the cold. The sodium salt was collected and recrystallized from a water-alcohol mixture; lemon-yellow needles; yield 29 Gm. (90%).

Anal.—Calcd. for $C_{11}H_9N_2O_4SNa$: Na, 7.98%. Found: Na, 7.94%.

10. Attempted Synthesis of 5-Acetamidoquinoline-8-sulfonamide Resulting in the Formation of 5-Acetamido-6-sulfamylquinoline-8-sulfonic Acid.—Chlorosulfonic acid (25 cc.) was cooled to 0° C. and 5 Gm. of sodium 5-acetamidoquinoline-8-sulfonate was added slowly with stirring. The mixture was gently boiled for eight minutes, cooled, and poured with stirring into ice water. The yellow precipitate which formed was filtered, washed with cold water and dried *in vacuo*. The dried powdered sulfonyl chloride was then added slowly with stirring to 10 cc. of 28% ammonia water and the stirring continued for one hour. The yellow solid which formed on cooling was dissolved in hot dilute alkali and the solution was made acidic with dilute hydrochloric acid; small yellow crystals separated. The properties and derivatives of the compound indicate it to be 5-acetamido-6-sulfamylquinoline-8-sulfonic acid. The benzylisothiourea salt was made in order to identify the compound.

11. Benzylisothiourea Salt of 5-Acetamido-6-sulfamylquinoline-8-sulfonic Acid.—This derivative was prepared analogous to a method described by Veibel and Lillelund (8) and by Urist (4). Lemon-yellow platelets were obtained; m. p. 232–233° C. (corrected).

Anal.—Calcd. for $C_{19}H_{20}N_5O_6S_2$: N, 13.70%. Found: N, 13.49%.

12. 5-Amino-8-chloroquinoline.—The method used was that described by Dikshoorn (9) with necessary modifications. To 2 Gm. of crude 5-nitro-8-chloroquinoline dissolved in 30 cc. of 50% acetic acid was added 3 Gm. of iron (20 mesh) over a period of two hours, during which time the suspension was heated to boiling and the stirring was continuous. The stirring and heating were continued for one hour longer after which the mixture was diluted to twice its volume with water, brought to boiling, made alkaline with 20% sodium hydroxide, filtered, and the filtrate placed in the cold. The precipitate recrystallized from water yielded 1.1 Gm. (65%) of product; m. p. 153–154° C.; Claus (10) reported 152° C.

13. 5-(*p*-Acetamidobenzenesulfonamido)-8-chloroquinoline.—Dry 5-amino-8-chloroquinoline (5 Gm.) was dissolved in 50 cc. of anhydrous pyridine in a small flask immersed in an ice bath. A trace of copper-bronze was added, and 6.5 Gm. of pure *p*-acetamidobenzenesulfonyl chloride was added in small portions with shaking. A reflux condenser holding a calcium chloride drying tube was attached and the flask placed on a steam bath for thirty minutes. The pyridine solution was diluted with about 1500 cc. of water, and the supernatant liquid was separated from the oily residue that formed and placed in the cold; colorless crystals separated. Additional crystals were obtained from the oily residue which was dissolved in alcohol, treated with decolorizing charcoal, filtered, water added to the precipitation point, and placed in the cold. The material was recrystallized by dissolving in alcohol, adding water to the precipitation point and placing in the cold; colorless needles, m. p. 264–265° C. with decomposition (corrected); yield 5.7 Gm. (54%).

Anal.—Calcd. for $C_{17}H_{14}N_3O_3SCl$: N, 11.19%. Found: N, 11.48%.

14. 5-(*p*-Aminobenzenesulfonamido)-8-chloroquinoline.—Dry 5-(*p*-acetamidobenzenesulfonamido)-8-chloroquinoline (2 Gm.) was refluxed for forty minutes with 40 cc. of 95% alcohol and 20 cc. of 16% hydrochloric acid. Sodium hydroxide (16%) was added to neutrality and on standing in the cold colorless needles separated. Recrystallized from 95% alcohol; yield 1.8 Gm. (practically quantitative); m. p. 233–234° C. with decomposition (corrected).

Anal.—Calcd. for $C_{15}H_{12}N_3O_2SCl$: N, 12.60%. Found: N, 12.76%.

15. 5,5′-Diamino-8,8′-diquinolyl Disulfide.—To a solution of 16 Gm. of stannous chloride in 15 cc. of concentrated hydrochloric acid was added slowly with cooling 3 Gm. of 5,5′-dinitro-8,8′-diquinolyl disulfide. The mixture was heated for forty-five minutes on a steam bath after which a yellow crystalline material separated. To the dry crystals was added sufficient 20% sodium hydroxide to make the mixture alkaline after which the free amine was dissolved in hot acetone. The hot solution was filtered, concentrated to one-fourth its volume water added to the precipitation point, and cooled. The crystals that formed were recrystallized by dissolving in hot acetone, adding water to the crystallizing point and cooling; yellow platelets, m. p. 225–226° C. with decomposition (corrected); yield 1.1 Gm. (44%).

Anal.—Calcd. for $C_{18}H_{14}N_4S_2$: N, 16.00%. Found: N, 15.88%.

16. 5,5′-Diacetamido-8,8′-diquinolyl Disulfide.—Impure 5,5′-diamino-8,8′-diquinolyl disulfide (0.6 Gm.) was refluxed for five minutes with 20 cc. of freshly distilled acetic anhydride. The precipitate that formed on cooling was recrystallized from alcohol; colorless needles, m. p. 285–286° C. with decomposition (corrected); yield 0.5 Gm. (70%).

Anal.—Calcd: for $C_{22}H_{18}O_2N_4S_2$: N, 12.90%. Found: N, 12.79%.

17. 5,5′-bis-(*p*-Acetamidobenzenesulfonamido)-8,8′-diquinolyl Disulfide.—To 2 Gm. of 5,5′-diamino-8,8′-diquinolyl disulfide dissolved in 24 cc. of anhydrous pyridine, were added, slowly with shaking, a trace of copper-bronze and 3.1 Gm. (10% excess) of pure *p*-acetamidobenzenesulfonyl chloride. The flask was attached to a condenser holding a calcium chloride drying tube, and the mixture heated one hour on a steam bath, diluted with about a liter of water, and placed in the cold. The precipitate that formed was recrystallized by dissolving in dilute alkali and acetone, and acidifying with dilute acid; light yellow platelets obtained from the precipitate began to darken at 275° C. and gradually turned black until they carbonized at 288° C.; yield 2.9 Gm. (69%).

Anal.—Calcd. for $C_{34}H_{28}O_6N_6S_4$: N, 11.29%. Found: N, 11.44%.

SUMMARY

1. Five new quinoline derivatives possessing possible medicinal value have been synthesized, namely; (*a*) 5-(*p*-aminobenzenesulfonamido)-8-chloroquinoline, (*b*) 5-(*p* - acetamidobenzenesulfonamido) - 8 - chloroquinoline, (*c*) 5,5′-diamino-8,8′-diquinolyl disulfide, (*d*) 5,5′-diacetamido-8,8′-diquinolyl disulfide, (*e*) 5,5′-*bis*-(*p*-acetamidobenzenesulfonamido) - 8,8′ - diquinolyl disulfide.

2. Sufficient quantities of these compounds have been prepared for bacteriological and pharmacological testing. The results of these tests will be published later.

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The Potency of a New Extract of *Convallaria majalis* Leaves. V. Studies on the Stability of Convara*,†,‡

By Harald G. O. Holck, Kazuo K. Kimura, and T. Eugene Kimura

The first assays upon a concentrated water-soluble extract of the leaves of *Convallaria majalis* Linne (Fam. *Liliaceae*), known as Convara, were carried out by the eighteen-hour frog method in 1941 (1). No deterioration was detected in samples stored over a period of five months under the following conditions: (*a*) without dilution in the refrigerator at 4° C., (*b*) without dilution at room temperature in the dark, and (*c*) as a 10 per cent aqueous solution at room temperature in the dark. In May and June of 1942, the first two cat assays, U. S. P. XII method (2), were conducted upon samples which had been stored in the refrigerator at 4° C. (3). In the first assay, the potency of Convara per 0.1 Gm. was equivalent to 8.6 ± 0.6 U. S. P. XI (1936) digitalis units while in the second assay 0.1 Gm. of Convara was equivalent to 11.8 ± 0.6 U. S. P. XII (1942) digitalis units.

The present studies were carried out more than a year later upon samples stored at room temperature in the dark and upon a 10 per cent solution similarly stored since the day we first received Convara, namely, November 18, 1940. The first objective was to ascertain by the U. S. P. XII cat method whether or not these samples had deteriorated. The second purpose was to determine whether or not we could obtain similar assay values for Convara when we used a concentration which would cause cardiac arrest in cats close to the lower limit specified by the U. S. P. XII (13 injections), and that producing cardiac arrest close to the upper limit (19 injections). The reason for assaying Convara at the two ends of the pharmacopœial limits was that convallaria preparations are shorter acting than digitalis (4, 5, 6, 7). The third purpose was to determine the effect of using pentobarbital sodium as an anesthetic instead of ether for the Convara assay with cats. The assay values with pentobarbital anesthesia for digitalis could be compared with the results from the digitalis assay using ether as the anesthetic. Also, comparisons could be made between the assays using pentobarbital anesthesia and those using ether anesthesia, first with the U. S. P. XII (1942) reference standard digitalis, and then with the U. S. P. XI (1936) reference standard.

The reference standard Tincture of Digitalis was made and diluted according to the U. S. P. XII, while Convara was diluted

* Received July 31, 1944, from the Department of Physiology and Pharmacology, College of Pharmacy, University of Nebraska, Lincoln, Neb.

† Convara, Lot No. 47888, was furnished by George A. Breon and Company, Kansas City, Mo.

‡ Aided by a grant from the same company.

prior to each assay with Isotonic Solution of Sodium Chloride, U. S. P. XII. Each cat was examined after the assay for gross evidence of abnormality and the females were checked for pregnancy.

RESULTS AND DISCUSSION

The results are presented in Table I. They show that when Convara is stored at room temperature in the dark, undiluted or as a 10 per cent aqueous solution, it remains remarkably stable. None of the results show any significant difference from those obtained with the sample stored in the refrigerator and assayed more than a year before. Thus, if the lowest value of 10.9 ± 0.7 units observed in Series C in Table I is compared with the original 1942 value of 11.8 ± 0.6 units in Series A, the critical ratio or the "t" factor calculated according to Burn (8) is only 1.0.

The stability of Convara is in harmony with previous reports on the stability of preparations made from parts of *Convallaria majalis* other than the leaves.

Although a 25 per cent difference in the concentration of Convara in Series B and C was employed to produce cardiac arrest near the ends of the pharmacopœial limits (13 and 19 injections, respectively), the relationship between the fatal dose of Convara and digitalis was constant. In Series B, the cats required 14.3 and 14.6 injections for Convara and digitalis, respectively; in Series C, 17.8 and 18.0 injections were necessary. Consequently, the digitalis units calculated for Convara were almost identical in case of the two concentrations used here.

In Series D, the value of 13.1 ± 0.9 digitalis units per 0.1 Gm. of Convara with pentobarbital anesthesia did not differ significantly from the 1942 value secured with ether anesthesia, since the "t" factor was only 1.2.

TABLE I.—ASSAYS UPON CONVARA KEPT UNDER DIFFERENT CONDITIONS OF STORAGE FOR VARYING LENGTHS OF TIME[a]

Series	Date of Assay	No. of Cats	Av. Weight, Kg.	Conc. of Drug and Storage Condition	No. of Injections	Mean Fatal Dose, Mg./Kg.	Standard Error as % of Mean	Digitalis Units per 0.1 Gm. of Convara
A	June 8–13, 1942	10	2.50	0.0526% Convara; refrigerator at 4°C.	13.6 ± 0.4	7.2 ± 0.2	3.2	11.8 ± 0.6
		10	2.60	5.6% Tr. of Digitalis	15.1 ± 0.6	84.6 ± 3.2	3.8	
B	July 3–16, 1943	6	2.58	0.050% Convara; room temperature in the dark	14.3 ± 0.3	7.2 ± 0.2	2.4	11.0 ± 0.4
		10	2.70	5.4% Tr. of Digitalis	14.6 ± 0.5	78.8 ± 2.6	3.3	
C	Sept. 25–Oct. 9, 1943	6	2.88	0.040% Convara; room temperature in the dark	17.8 ± 1.0	7.1 ± 0.4	5.5	10.9 ± 0.7
		6	2.76	0.040% Convara; 10% sol.; room temperature in the dark	17.3 ± 0.9	6.9 ± 0.4	5.3	11.2 ± 0.7
		7	2.77	4.32% Tr. of Digitalis	18.0 ± 0.6	77.8 ± 2.7	3.4	
D[b]	Oct. 27–Dec. 11, 1943	11	2.50	0.064% Convara; room temperature in the dark	16.1 ± 0.7	10.3 ± 0.5	4.5	13.1 ± 0.9 Pentobarbital anesthesia in Series D
		14	2.56	7.7% Tr. of Digitalis[c]	17.6 ± 0.8	135.2 ± 6.2	4.6	

[a] Unless otherwise specified, the assays were carried out according to the U. S. P. XII cat method.
[b] This assay was aided by a grant from S. B. Penick & Company, New York, N. Y.
[c] We are indebted to Prof. E. Fullerton Cook for part of the Reference Standard Digitalis.

In the assays with pentobarbital anesthesia, more cats were used than actually included in Table I, where only the cats weighing between 2.0 and 4.0 Kg., as specified by the Pharmacopœia, were considered. With Convara, a total of 17 cats weighing between 1.61 and 4.12 Kg. (av. 2.50 Kg.) were assayed and the average number of injections required was 15.4 ± 0.5, equivalent to 9.9 ± 0.3 mg./Kg. With digitalis, a total of 20 cats weighing between 1.73 and 4.14 Kg. (av. 2.56 Kg.) were assayed with pentobarbital anesthesia. These required on an average 16.0 ± 0.6 injections, equivalent to 130.1 ± 4.8 mg./Kg. These figures give a value of 13.2 ± 0.7 units per 0.1 Gm. of Convara, a value almost identical with 13.1

± 0.9 units shown in Table I for cats weighing between 2.0 and 4.0 Kg., respectively, as specified by the United States Pharmacopœia.

Under pentosbarbital anesthesia, the cats were more resistant to both Convara and digitalis. Thus, with Convara the ratio is 9.88/7.16 or 1.38, a difference of 38 per cent. With digitalis the ratio is 130.1/84.6 or 1.54, a difference of 54 per cent. The latter checks well with the 47 per cent difference obtained with a much greater series of cats using the continuous injection technique (9,10) and the 1936 reference standard Tincture of Digitalis (U. S P. XI).

SUMMARY

1. No deterioration of Convara was demonstrable in an undiluted or in a 10 per cent solution of this water-soluble extract of the leaves of *Convallaria majalis* kept in the dark at room temperature over a period of several years as judged by assays according to the U. S. P. XII cat method for digitalis.

2. The relation between the fatal dose of Convara and of digitalis was constant whether the concentration used in the assay caused cardiac arrest in cats with fourteen and a half or eighteen injections for both drugs.

3. When pentobarbital sodium was used as the anesthetic, the number of digitalis units per 0.1 Gm. of Convara did not differ significantly from those obtained with ether anesthesia. With Convara and digitalis, the cats required 38 per cent and 54 per cent more drug, respectively, to produce cardiac arrest with pentobarbital than with ether anesthesia.

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Adulterated Arnica Flowers*

By Clare Olin Ewing† and Albin Stitt‡

During the last war, adulteration of imported drugs was rather widespread (1). Adulteration during the present war does not appear to be so prevalent, owing in part to greater use of synthetic drugs, in part to greater use of domestic wild growing or cultivated drugs and possibly in part to two decades of additional education of growers and importers by the U. S. Food and Drug Administration.

Nevertheless several instances have come to our attention that should be made a matter of record to protect other users of these products. One of these instances is an adulterant of Arnica Flowers, N. F., *Arnica montana* L. A shipment of three bales proved to consist entirely of the flower heads of *Inula britannica* L. Since both species develop an abundant pappus, they bear superficially some resemblance to the flower heads of the official *Arnica montana* L., although they are somewhat smaller. They are practically devoid of odor even when broken up by rubbing between the palms of the hand. Closer examination shows several conspicuous and distinguishing characteristics. These are listed in Table I and illustrated (Fig. 1) through the courtesy of our colleague, Mr. E. F. Tilc.

Most of the commercial supply of Arnica Flowers, N. F., normally reaching the United States is collected in Germany and Yugoslavia. The flowers have been so

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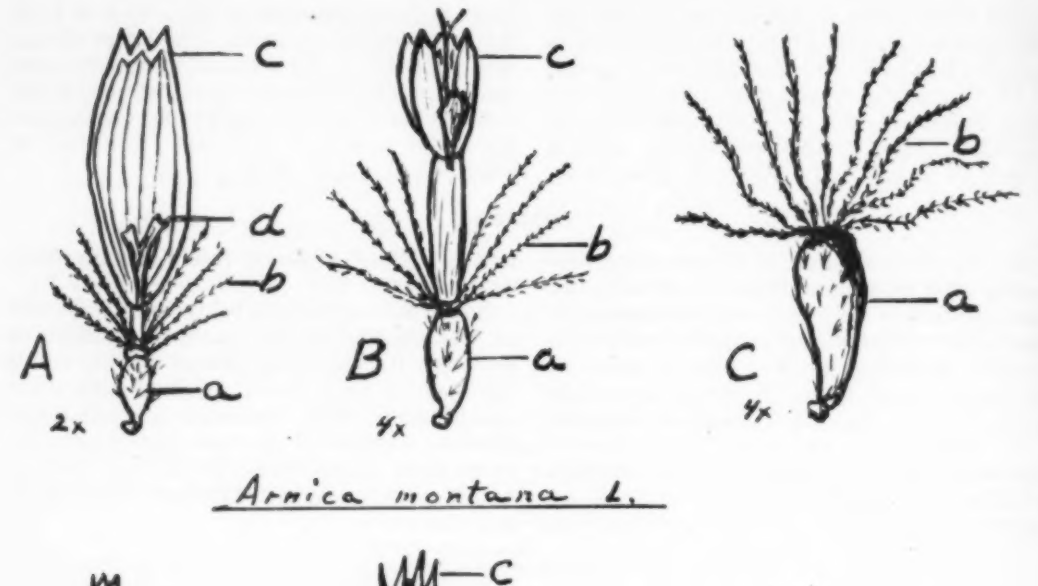

Fig. 1.—Differentiating characteristics between *Arnica montana* L. and *Inula britannica* L. *Arnica montana:* *A*, ligulate ray floret (2×); *B*, tubular disk floret (4×); *C*, achene (4×). *Inula britannica:* D, ligutate floret (4×); E, tubular disk floret (7×); F, achene (10×). *a*, achene; *b*, pappus bristles; *c*, apex of floret; *d*, bifid stigma; *e*, tubule of disk floret.

TABLE I.—PRINCIPAL DISTINGUISHING CHARACTERISTICS BETWEEN *Arnica montana* L. AND *Inula britannica* L.

	Arnica montana L.	*Inula britannica* L.
Ray flowers (ligulate)	7–12 veined, up to 25 cm. long	4 veined, about 1 cm. long
Disk flowers (tubular)	About 1.5 cm. long, 5 toothed at apex	About 0.9 cm. long, 5 toothed at apex
Achene	5–7 mm. long, pubescent, 5–10 ribbed, finely striate	1–1.5 mm. long, glabrous, 4–5 ribbed

difficult to obtain that the appearance of *Inula britannica* L. as an adulterant is not surprising. Curiously enough, the same adulterant appeared on the market during World War I. This is the only instance that has come to our attention where the same adulterant of an imported drug has been offered for sale during both wars.

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